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Molecular and biochemical analysis of the gelatinization temperature characteristics of rice (*Oryza sativa* L.) Starch granules

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Abstract:

Through integrated molecular and biochemical investigations and by using a common mutant line in molecular mapping, we have shown that the SSIIa gene, previously reported to be responsible for the starch granule gelatinization temperature (GT) differences between *indica* and *japonica* rice varieties, might also control the GT variations among certain *indica* varieties. This effect is mediated through differences in the amounts of SSIIa protein bound to starch granules leading to differences in the structure of amylopectin molecules synthesized. For the first time it was shown, that the amylopectin type accounted only for the GT differences between varieties with different SSIIa alleles, but not between varieties carrying a common SSIIa allele. In the latter case, another gene, *alk2(t)*, with a genetic distance of 3.93 cM from the *Wx* gene, was identified as being responsible for the GT variations. Based on the thermal properties and amylopectin chain length profile characteristics, it is postulated that the SSIIa gene has at least three different alleles, one in *japonica* rice and two in *indica* rice varieties, whereas *alk2(t)* has at least two alleles for either low or high GT. Thus, the rich diversity of the GT character in rice very probably results from various combinations of these alleles.

Keywords: Gelatinization temperature; Amylopectin chain length; Starch synthase gene IIa (SSIIa); Gene mapping; Microsatellite marker; *Oryza sativa* L

1. Introduction

The gelatinization temperature (GT) is the temperature at which starch granules irreversibly lose their crystalline order during cooking (Parker and Ring, 2001) and is the most important cooking quality characteristic of rice grains (Juliano, 1985). In practical quality evaluation and in most research experiments, GT is usually measured indirectly according to the digestibility of milled rice grains in an alkaline solution and scored by the alkali spreading value (ASV), since the disintegration of rice grains in alkaline solutions is closely associated with their cooking properties (Little et al., 1958) and the GT of milled rice (Juliano et al., 1964). More sophisticated analysis of the thermal characteristics of rice grains can be made using a differential scanning calorimeter (DSC), which allows the determination of the temperature at which starch granule gelatinization is initiated and completed, as well as the heat energy required for gelatinization (Marshall, 1994).

It has been well documented through classical genetic analysis (Hue and Choi, 1973; McKenzie and Rutger, 1983; Umemoto et al., 2002) and molecular mapping studies (Bao et al., 2004; He et al., 1999; Tian et al., 2005; Umemoto et al., 2002; Yan et al., 2001) that a single major gene, designated *alk* and mapped on chromosome 6 (Kudo, 1968), is responsible for the ASV variations in *indica* and *japonica* crosses. However, in the progenies derived from crosses of the same subspecies, the mode of inheritance appeared very

Abbreviations: AAC, apparent amylose content; ACL, amylopectin chain length; ACR, ratio of short chains with degree of polymerization (DP) ≤ 10 to short and intermediate chains with DP ≤ 24; ASV, alkali spreading value; CMS, cytoplasmic male sterility; DP, degree of polymerization; DSC, differential scanning calorimetry; GT, gelatinization temperature; PCR, polymerase chain reaction; QTL, quantitative trait locus; SSIIa, starch synthase IIa; SSR, simple sequence repeat.

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complicated and sometimes contradictory. Ghosh and Govindasvamy (1972) reported that ASV was quantitatively inherited, whereas McKenzie and Rutger (1983) found that the segregation patterns in five crosses did not conform to any identifiable genetic model. Quantitative trait locus (QTL) mapping results also were not consistent when different *indica*×*indica* populations were used and were different from *indica*×*japonica* crosses. For example, Tan et al. (1999) mapped the major QTL for ASV variation in the chromosomal region of the waxy (*Wx*) gene rather than the *alk* locus, although the same group later mapped the major QTL for ASV onto the *alk* locus in a doubled haploid population derived from a cross between two *indica* varieties (Fan et al., 2005). Umemoto et al. (2002) attributed the different mapping results to differences in experimental materials, but no clear genetic or biochemical basis was provided to explain the inconsistencies.

More recently GT variations among waxy varieties measured using DSC, were shown to be closely related to the structural differences in the amylopectin of the starch (Patindol and Wang, 2002; Qi et al., 2003; Shi and Seib, 1992). Based on the finding that the starch synthase gene IIa (SSIIa) and the genes that control the amylopectin chain length (ACL) profile and alkali disintegration in rice, mapped to the same locus as the *alk* gene, Umemoto et al. (2002) suggested that the differences in alkali digestibility between *indica* and *japonica* rice subspecies might be controlled by the SSIIa gene. Most recently this was further supported by the finding that a few essential amino acids in SSIIa determine the difference in the amylopectin structure and the GT between *indica* and *japonica* rice (Nakamura et al., 2005). Clearly then the genetic and biochemical basis of the GT character in rice, particularly in the *indica* subspecies where GT variations are common, is not fully understood.

Induced mutation has become an important source of genetic variations in plant breeding programs (Ahloowalia et al., 2004). According to the FAO/IAEA Mutant Variety Database (http://www-mvd.iaea.org/MVD/default.htm), more than 2400 mutant varieties including about 450 rice varieties have been developed worldwide during the past 40 years. Further, more mutant lines have been developed for functional genomic studies and for mutational analysis of important biological processes in plants (Kurata et al., 2005). In the present study, an artificially induced, low GT mutant, together with its parent and other materials with different thermal properties, was investigated in an attempt to uncover additional genetic and biochemical control mechanisms for the GT character in rice.

2. Experimental

2.1. Plant materials

The mutant rice line, cv. Huangyu B, with an ASV of 6-7, was developed through gamma irradiation of an *indica* rice variety, cv. II32 B with an ASV of 2.0 (Shu et al., 2001). Huangyu B was recurrently backcrossed to the cytoplasmic male sterile (CMS) line II32 A (the corresponding CMS line of II32 B), and a new CMS line Huangyu A was developed (Zhou et al., 2006). Other rice varieties used in the experiments included the *indica* rice varieties Niqingzhanand R3027, IR36 and *japonica* Xiushui 110 and Kinmaze.

2.2. GT evaluation

Both alkaline disintegration (Little et al., 1958) and DSC methods were used in GT measurements. In the alkali disintegration test, milled rice grains (10 each for homozygous varieties and 24 each for segregating progenies, i.e. seeds from individual F_2 plants) were treated in 1.7% KOH solution at 30 °C for 23 h, and the ASV was recorded on a 2–7 scale. DSC measurements of thermal properties was made on a thermal analyzer (DSC-7, Pekin–Elmer, USA). Rice grains were dehulled using a Satake Dehuller (Satake Corporation, Japan), milled using a Satake test mill (Satake Corporation, Japan), and ground into flour using a cyclone sample mill (UDY Corporation, USA). Rice flour (1.8 mg) was weighed onto an aluminum pan, mixed with 12 μl of distilled water, and sealed. The pan was heated at a rate of 10 °C/min from 30 to 110 °C. Another sealed pan with 12 μl of distilled water was used as a control. The onset (T_o), peak (T_p) and conclusion (T_c) temperature of gelatinization were calculated automatically using the Universal Analysis Program, Version 1.9D.

2.3. Genetic analysis of the GT character

F_1 hybrid seeds were produced by artificial crossing. The segregating populations of Huangyu B/II32 B and Huangyu B/R3027 were developed through self-pollination of F_1 and F_2 plants. All materials were transplanted at a single seedling per hill and grown at the experimental farm at the Huajiachi campus of Zhejiang University from 2001 to 2004. The seed samples used in each analysis were harvested from the same field and in the same season to minimize possible environment effects.

F_1 seeds of each cross were tested by alkali disintegration and the DSC method. For segregation analysis, $F_{2:3}$ seeds were harvested on an individual plant basis from F_2 plants of Huangyu B/II32 B and Huangyu B/R3027. Twenty-four $F_{2:3}$ seeds of each F_2 plants were analyzed for the ASV. The F_2 plants were then classified into three types: homozygous, low ASV (all $F_{2:3}$ seeds had a low ASV of <3), homozygous, high ASV (all $F_{2:3}$ seeds had a high ASV of >4) and heterozygous ASV ($F_{2:3}$ seeds had ASV ranging from 2 to 6 or 7).

2.4. Molecular mapping

The two F_2 populations, Huangyu B/II32 B and Huangyu B/R3027, were used for molecular mapping of loci responsible for alkali digestibility. Genomic DNA was extracted using leaf tissues of F_2 plants following a modified CTAB method (Lu and Zheng, 1992). DNA samples were quantified using a Unican UV300 (Thermo Electron Corporation, Cambridge, UK) and adjusted to a final concentration of about 25 ng/μl.